Comparison of cortisol, luteinizing hormone, and testosterone responses to a defined stressor in sexually inactive rams and sexually active female-oriented and male-oriented rams[1]

J. N. Stellflug[2]

USDA-ARS US Sheep Experiment Station, Dubois, ID 83423

ABSTRACT: The objective of this study was to determine whether the effect of restraint stress on cortisol, LH, and testosterone varied among sexually inactive and sexually active female- and male-oriented rams, to allow differentiation among ram classes. Restraint stress or no stress was imposed on sexually inactive (n = 7) and sexually active female- (n = 17) and male-oriented (n = 6) rams in a 2 × 3 factorial arrangement. Rams were assigned to restraint or control within each classification. Rams were habituated to wearing halters and being tethered in separate pens, permitting visual, vocal, and olfactory contact with adjacent rams for 7 d before treatment. After 1 d of habituation, rams were fitted with jugular catheters that were checked twice daily for patency. For restraint stress, rams were laid on their side with their legs tied for 1 h. For no stress, rams were tethered with halters and leads, but their legs were not tied. On the treatment day, blood was collected at 30-min intervals for 3 h followed by 15-min intervals for 1 h before restraint, during 1-h restraint, and for 1 h after liberation from restraint. Then blood was collected at 30-min intervals for an additional 2 h. Blood was collected from controls at similar intervals. Control rams were isolated from stressed rams. Cortisol, LH, and testosterone were measured using RIA. Mixed model analyses with repeated measures were used on transformed data. Average prestress data were used as a covariate. Cortisol increased ($P < 0.01$) within 15 min after restraint and remained increased until 1.5 h after liberation from 1-h of restraint stress. In contrast, in controls cortisol remained unchanged at 5 ng/mL. Cortisol did not differ over time among ram classes, and the treatment × ram class × time interaction was not significant. For LH and testosterone, the ram class × time interactions appeared to compromise the ability to identify differences in these hormones, indicating that they were not good endocrine candidates for methods of classifying rams. In conclusion, restraint stress increased cortisol in sexually inactive and sexually active female- and male-oriented rams alike, thus not providing a method to differentiate among ram classes.

Key words: cortisol, luteinizing hormone, ram, sexual performance, stress, testosterone

©2006 American Society of Animal Science. All rights reserved. J. Anim. Sci. 2006. 84:1520–1525

INTRODUCTION

Rams with distinctly different sexual behaviors do not have overt phenotypic characteristics or basal systemic concentrations of LH, testosterone, or estradiol (Alexander et al., 1993) that differentiate them. However, there are distinct differences among classes of rams in their brains and in their hormonal responses to various stimuli that may lead to an efficient means of identifying ram classes.

Differences exist in brains from various ram classes: volume of ovine sexually dimorphic nucleus and level of aromatase expression within this nucleus differ for female- and male-oriented rams and ewes (Roselli et al., 2004); estrogen receptor content in amygdala of male-oriented rams is similar to the ewe and less than that for female-oriented rams (Perkins et al., 1995).

Differences exist in hormonal response to various stimuli among ram classes: high sexual performance rams respond with increased LH and testosterone to prolonged exposure to estrual ewes, unlike low sexual performance and male-oriented rams (Perkins et al., 1992); LH and testosterone increases to naloxone were earlier and testosterone was greater in sexually active than inactive rams (Stellflug et al., 2004); under anesthesia-induced stress, testosterone concentrations were greater and cortisol lower in female-oriented than in male-oriented and asexual rams (Roselli et al., 2002), suggesting that increased stress responsiveness may be correlated with decreased libido.

[1]Acknowledgments: V. A. LaVoie is cordially thanked for excellent technical assistance and B. E. Mackey for statistical advice.

[2]Corresponding author: jstellflug@pw.ars.usda.gov

Received July 28, 2005.

Accepted January 12, 2006.

Given these differences and that there are varied responses to stress between sexes (Tillbrook et al., 1999), the working hypothesis was that rams with different behavioral classes should respond differently to stress to allow differentiation of ram classes. Thus, the objective for this study was to determine whether a reliable stressor (restraint) would differentially affect cortisol, LH, and testosterone in sexually inactive, sexually active female-oriented, and male-oriented rams allowing for separation among ram classes.

MATERIALS AND METHODS

General

Rams and ewes were kept at the USDA-ARS US Sheep Experiment Station, Dubois, ID, located at 44° 14′N and 112° 11′W. The USDA-ARS US Sheep Experiment Station Animal Care and Use Committee approved all animal protocols. All animals were offered long-stem alfalfa hay (relative feed value of 165) at 2.2% of BW (DM basis) daily and were given free access to water and trace mineral salt [Redmond T.M. (2,000 ppm of Mn; 3,500 ppm of Zn; 600 ppm of Fe; 300 ppm of Cu; 80 ppm of I; and 50 ppm of Co), Redmond Minerals Inc., Redmond, UT] in outside paddocks. The ewes were ovariectomized and estrus-induced using 60-mg 6α-methyl-17α-hydroxyprogesterone acetate pessaries (Pharmacia and Upjohn, Kalamazoo, MI) and injection of 50 μg of estradiol 17β, as explained in detail previously (Stellflug and Berardinelli, 2002). Ewes were considered to be in estrus when they would stand to be mounted by a ram.

Sexual Performance Tests

Thirty rams [2- to 3-yr old Targhee (n = 13), Polypay (n = 7), and Rambouillet (n = 10)] were selected based on 3 types of sexual performance tests: 1) a preliminary screening test, in which rams were observed for 30 min with 3 estrus-induced ewes (Snowder et al., 2002), and rams were initially classified as sexually inactive (no ejaculations) or active (1 or more ejaculations); 2) a series of nine 30-min serving capacity tests (Stellflug and Berardinelli, 2002), in which rams were observed with 3 estrual ewes; and 3) four 30-min sexual partner preference tests (Stellflug and Berardinelli, 2002) in which rams were observed with 2 restrained estrual ewes and 2 restrained rams. Rams were classified as sexually inactive, sexually active with ewes (female-oriented), or as exclusively sexually active with rams (male-oriented). Sexually inactive rams had no ejaculations per test; sexually active female-oriented rams had a range of 2.6 to 3.6 ejaculations per test; and male-oriented rams had a range of 3 to 21 mounts per test on rams, for an average of 9.0 mounts per test.

Habituation and Administration of Restraint Stress Test

Rams were habituated to wearing halters, being tethered, and standing in separate pens. Rams had visual, vocal, olfactory, and tactile contact with a neighboring ram for 7 d before administering stress treatments in early October (during the breeding season). After 1 d of habituation, all rams were fitted with indwelling jugular catheters to minimize stress associated with blood collection. Habituation included checking patency of the catheters in the morning and afternoon daily.

Rams were randomly assigned to 2 stress treatments within ram class (sexually inactive, sexually active female-oriented, or sexually active male-oriented) in a 2 × 3 factorial arrangement. Treatments consisted of either control (nonstressed) or restraint stress. Control rams were tethered with halters and leads, but not tied down, remained in individual habituation pens for the 8-d period, and were isolated from stressed rams in the opposite wing of the barn. Control rams had the freedom to stand or lie down throughout the blood collection procedure. Both wings of the barn are set up identically to each other. Restraint-stressed rams were tethered with halters and leads and remained in individual habituation pens. For the 1-h restraint period, rams were laid on their side with their front legs tied together and their hind legs tied together with separate hobbles. Hobbles (46.2 × 2.6 cm nylon straps with buckles) were used to reduce abrasion and allow consistent tightness of restraint. The final distribution of the rams to treatments was 7 sexually inactive rams (4 controls and 3 stressed), 17 female-oriented rams (8 controls and 9 stressed), and 6 male-oriented rams (3 controls and 3 stressed).

Blood Collection Procedure

Blood samples were taken via indwelling jugular catheters (16 gauge, 1.7 × 133 mm Angiocath, Becton Dickinson, Sandy, UT). Samples were collected at 30-min intervals for 3 h before treatment, at 15-min intervals for 1 h before and for 2 h after the onset of treatment, followed with 30-min intervals for an additional 2 h after treatment, for a total of 8 h. Blood was collected from control rams at the same times it was collected from stressed rams. The blood collection intervals were chosen based on the collection protocol used for previous studies with naloxone (Stellflug, 2002; Stellflug et al., 2004), with the exception of longer sampling periods before and after treatment to monitor cortisol and testosterone, which were the 2 main hormones of interest for this study. The collection intervals were not designed to evaluate LH pulse characteristics, which requires sampling approximately every 6 min for 10 to 12 h and the use of the MUNRO (Ji et al., 1989) or Cluster algorithms (Veldhuis and Johnson, 1986). The blood was drawn with 6-mL syringes and transferred into 5-mL glass tubes containing 2 drops of heparin (50 USP units of H3393 porcine heparin/mL of physiological saline; Sigma, St. Louis, MO). Plasma was harvested and stored at –20°C until cortisol, LH, and testosterone concentrations were measured.

Hormone Assays

Plasma concentrations of cortisol were quantified using RIA kits (Diagnostic Products Corp., Los Angles, CA). The intra- and interassay CV were less than 9.5 and 12.6%, respectively, for 5 assays, with a sensitivity of 0.02 ng/mL.

Plasma LH concentrations were quantified using a validated RIA procedure (Perkins et al., 1992) that included anti-oLH AFP-192279 and oLH AFP-8614B for iodination and standards, which were obtained through the National Hormone Pituitary Program of the National Institute of Diabetes, Digestive, and Kidney Diseases. The intra- and interassay CV were less than 7.1 and 10.5%, respectively, for the 2 assays, with a sensitivity of 0.25 ng/mL. The value for sensitivity of the assay was used for samples that were less than the detection limit.

Plasma concentrations of testosterone were quantified using RIA kits (Diagnostic Products). Cross-reactivity was 3.3% with 5α-dihydrostestosterone. The intra- and interassay CV were less than 10.9 and 6.4%, respectively, for 5 assays, with a sensitivity of 0.04 ng/mL.

Statistical Analyses

Cortisol, LH, and testosterone data were analyzed using mixed model procedures of SAS (SAS Inst. Inc., Cary, NC) for specific repeated measures. The cortisol and testosterone data were evaluated for all time periods before treatments were administered and all time periods after onset of treatment, including the time just before treatment, in separate analyses for each hormone. The LH data only included the values from samples collected at 15-min intervals, with the pre- and post-treatment samples evaluated in separate analyses. Corresponding average pretreatment values were used as a covariate in the after treatment analyses. The main plot included terms for ram class (sexually inactive, sexually active female-oriented, or sexually active male-oriented rams), treatment, and ram class × treatment. The subplot included sample time, ram class × sample time, treatment × sample time, and ram class × treatment × sample time. Ram class and treatment were tested with rams nested within ram class and treatment. The subplot was tested with the residual. Degrees of freedom were calculated using the Kenward-Roger procedure (Kenward and Roger, 1997). The first-order autoregressive moving-average command was used to structure the covariance matrix.

Data for cortisol, LH, and testosterone showed heterogeneous variance using Bartlett's Box F-Test. To normalize variances among rams for fixed effects, cortisol and LH values were transformed to the natural logarithm, and testosterone values were transformed to the inverse square root. Cortisol, LH, and testosterone least squares means and confidence intervals were changed back to their original units after analysis. A SE for the original units was estimated using the 95% confidence intervals; this is an approximation (approximate SE) and not appropriate for estimating confidence intervals for means that would asymmetrically match the data distribution. If the main effects or their interactions were significant ($P < 0.05$), Fisher's protected LSD was used as a postanalysis test for mean separation.

In addition, the testosterone profiles were evaluated with algorithms by using the Pulse XP programs (Veldhuis and Johnson, 1986). Area under the curve and number of peaks before and after stress treatment were calculated for testosterone. Data from the Pulse 2 algorithms were used for Deconvolution algorithm analyses (Veldhuis et al., 1987). Values for area under the curve had heterogeneous variance using Bartlett's Box F-Test and were transformed to the natural logarithm to normalize variances before analysis with the mixed model procedures of SAS. The model included terms for ram class, treatment, and ram class × treatment with ram within ram class as a random effect. The number of testosterone peaks before and after stress treatments was analyzed with Glimmix procedures of SAS. Number of peaks over 1 was recorded as 1 peak for a binomial distribution because of limited incidence. The model included terms for treatment and period of peaks (before and after stress treatments) and treatment × period with ram within treatment as a random effect. Tests of simple effects were provided between periods for each treatment.

RESULTS

During the 4 h before treatments, cortisol only varied ($P < 0.05$) over time, reflecting acclimation to the sampling regimen. Briefly, there was an increase in cortisol from 3.1 ± 0.6 ng/mL to 5.0 ± 0.9 ng/mL 30 min after the first sample followed by a fluctuation of cortisol concentrations around 2 to 4 ng/mL until an increase at time 0 (4.6 ± 0.9 ng/mL) just before treatment. The variation in cortisol concentrations before treatment was accounted for with the use of a covariate that indicated ($P < 0.001$) improved precision of the test for fixed effects after treatments. Cortisol increased ($P < 0.01$) within 15 min after onset of restraint in the stressed rams and remained increased until 1.5 h after the end of the 1-h restraint stress (Figure 1). In contrast, cortisol remained unchanged at approximately 5 ng/mL in control rams. Cortisol did not differ over time among the ram classes, and the treatment × ram class × time interaction was not significant.

Before treatments, LH profiles for sexually inactive stressed rams and male-oriented control rams intersected with sexually active control rams at 15 min before treatment as indicated with the treatment × ram class × time interaction ($P < 0.05$). Even though the variation in LH before treatments did not result in a significant covariate in the separate analysis after treatments, the interaction before treatments suggests

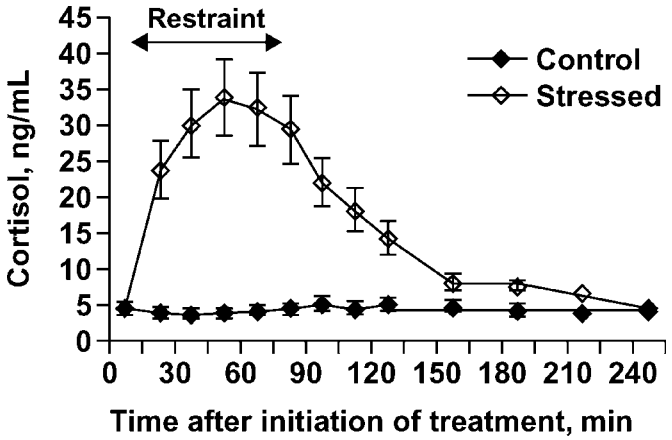

Figure 1. Least squares means for cortisol for 4 h after the onset of stress treatment (n = 15 controls, or n = 15 restraint stress) that were administered at time zero in a 2 × 3 factorial arrangement. Average prestress data as a covariate accounted for some of the variability ($P < 0.001$) and strengthened the tests for variables and interactions in the main plot and subplot. Cortisol increased ($P < 0.01$) within 15 min after the onset of restraint stress for all 3 ram classes (sexually inactive, sexually active female-oriented, and sexually active male-oriented rams) and remained elevated for 1.5 h after restraint ended at 60 min after the onset of treatment, compared with low concentrations of cortisol throughout the 4-h period in control rams from each ram class; only these data are depicted in the figure. The treatment × ram class × time interaction was not significant. The estimate of variability is depicted by estimated SE of the least squares means because of data transformation.

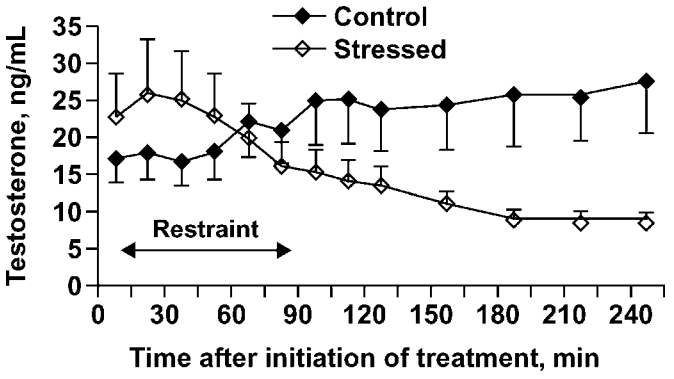

Figure 2. Least squares means for testosterone for 4 h after treatments were administered at time zero in a 2 × 3 factorial arrangement (n = 15 controls, or n = 15 restraint stress). Testosterone was stable in controls over time at 18 to 25 ng/mL, whereas testosterone began decreasing at 1.5 h and remained decreased to 4 h after the onset of the restraint stress in all ram classes, as indicated by the treatment × time interaction ($P < 0.05$). The estimate of variability is depicted by estimated SE of the least squares means because of data transformation.

that LH was not a good candidate endocrine measure for selection/screening of rams.

Before treatments, testosterone concentrations varied at random with intersecting lines for each ram class and as indicated with the treatment × ram class × time interaction ($P < 0.001$). Even though the variation in testosterone concentrations before treatment was accounted for with the use of a covariate, the interaction suggests that testosterone was not a good candidate endocrine measure for selection/screening of rams.

After onset of treatments, testosterone concentrations varied at random with intersecting lines for each ram class as indicated with the ram class × time interaction ($P < 0.05$). Testosterone remained stable at 18 to 25 ng/mL in controls over time, whereas testosterone began decreasing at 1.5 h after onset of stress and remained decreased out to 4 h after onset of the restraint stress for all ram classes combined as shown in Figure 2, and indicated with the treatment × time interaction ($P < 0.05$).

Area under the curve for testosterone for the period after onset of stress treatments differed ($P < 0.01$) for treatment but not for ram class and did not differ before stress treatments. Tests between periods for each treatment indicated that LSM for number of peaks differed ($P < 0.01$) for stressed rams before (0.8 ± 1.0) versus after (0.4 ± 1.4) stress onset. Incidence of number of peaks before and after the time of treatments did not differ for control rams.

DISCUSSION

For this study, restraint stress was used to determine whether sexually inactive rams or sexually active female-oriented or male-oriented rams on a multiple sampling regimen had different responses in cortisol, LH, and testosterone concentrations that would allow differentiation among ram classes. Restraint stress for 1 h stimulated an immediate cortisol increase similarly in sexually inactive rams and sexually active female-oriented and male-oriented rams. This is similar to results from a study using transportation stress (C. E. Roselli, J. N. Stellflug, and F. Stormshak, Portland, OR, unpublished data); however, it is in contrast to an earlier report that a combination of anesthesia and immobilization stress resulted in lower systemic concentrations of testosterone and greater concentrations of cortisol in sexually inactive or male-oriented rams compared with high sexual performance, female-oriented rams (Roselli et al., 2002). In that study, it was not possible to determine whether the outcome could be attributed to a direct pharmacological action of anesthetic agents on the central nervous system or to acute stress induced by the physiological reaction to anesthesia and immobilization (Roselli et al., 2002). This study with restraint stress and the study with transportation stress (C. E. Roselli, J. N. Stellflug, and F. Stormshak, Portland, OR, unpublished data) indicate that stress alone did not result in

a differential response in cortisol that was related to sexual behavior classification of rams. The interactions of ram sexual behavior classifications with time before restraint were obvious in evaluations of LH and testosterone, suggesting that they were not good endocrine candidates for classifying rams. This is probably related to low numbers of sexually inactive and male-oriented rams preventing any conclusions about whether differences in LH and testosterone existed among these classes, but if differences do exist the magnitude is not sufficient to allow for these endocrine variables to serve as methods of classifying the rams. Thus the hypothesis that rams with different behavioral classes should respond differently to stress and be a useful means to differentiate among the 3 classes of rams was not verified based primarily on similar cortisol responses for the classes of rams after restraint stress.

The multiple sampling regimen for 4 h before the stress treatments further substantiates the information from other studies (D'Occhio and Brooks, 1982; Alexander et al., 1999; Pinckard et al., 2000) that variations in basal testosterone concentrations do not seem to have a major relationship to sexual performance differences observed in adult rams. This concept is supported in studies with guinea pigs (Harding and Feder, 1976) and rats (Whalen et al., 1961) in which no differences in circulating testosterone were found between sexually active and inactive males.

The current study was not specifically designed to address the LH and testosterone profile relationship in rams that has been well established (Sanford et al., 1974; Wilson and Lapwood, 1979; Haynes and Schanbacher, 1983). During the 15-min sample collection period initiated 1 h before treatment and continuing for 2 h after treatment, there were only a few LH increases in the individual ram data observed; this is not uncommon for this short a period of observation.

Testosterone concentration is known to increase in the breeding season (Schanbacher and Lunstra, 1976) and is probably the reason for the relatively high testosterone before stress in the current study. The immediate increase in cortisol and the subsequent decrease in testosterone concentration beginning at 1.5 h after onset of restraint stress and remaining through at least 4 h after the initiation of restraint in rams is similar to what has been reported for immobilized rats (Orr and Mann, 1990) and after stress for bulls (Welsh and Johnson, 1981; Welsh et al., 1999) and rams (Juniewicz et al., 1987). The decrease in testosterone concentration after stress is also supported with the decrease in number of testosterone peaks observed after stress.

Corticoids are thought to affect the hypothalamic-pituitary-gonadal axis by suppressing LHRH secretion in rams (Juniewicz et al., 1987). Others have suggested that hormones from the adrenal axis suppress the LH response to LHRH at the level of the pituitary in rams (Matteri et al., 1984). Suppression at the level of the pituitary is supported by recent experiments with ovariectomized ewes during the anestrous period (Breen and Karsch, 2004). Stackpole et al. (2003) also reported that in sheep, stress decreases response to GnRH at the level of the pituitary during anestrus but not during the breeding season and possibly through mediators of the stress response other than those from the hypothalamic-pituitary-adrenal axis. However, a possible direct effect of corticoids on testosterone at the level of the testes cannot be ignored based on data for rats (Welsh et al., 1982; Rivier and Rivest, 1991), and decreases in testosterone do not have to be associated with decreases in LH, as observed for rats (Orr et al., 1994). Further research is needed to investigate the mechanisms by which stress decreases reproduction, but it is not readily apparent that this will lead to a more practical method for identifying different sexual behavioral classes of rams.

In conclusion, even though rams of different sexual activity classes have differential hormonal responses to various stimuli such as estrual ewes (Perkins et al., 1992) and naloxone (Stellflug, 2002), restraint stress alone does not induce differential responses in cortisol in sexual classes of rams. The minimal number of rams in some classifications prevented conclusions about whether differences in LH and testosterone existed among classes of rams, but if differences exist the magnitude would not be sufficient to allow these endocrine variables to serve as methods of classifying the ram. Thus, the cortisol response to restraint stress does not provide a means of differentiating among sexually inactive or sexually active female- or male-oriented rams.

LITERATURE CITED

Alexander, B. M., A. Perkins, E. A. Van Kirk, G. E. Moss, and J. A. Fitzgerald. 1993. Hypothalamic and hypophyseal receptors for estradiol in high and low sexually performing rams. Horm. Behav. 27:296–307.

Alexander, B. M., J. N. Stellflug, J. D. Rose, J. A. Fitzgerald, and G. E. Moss. 1999. Behavior and endocrine changes in high-performing, low-performing, and male-oriented domestic rams following exposure to rams and ewes in estrus when copulation is precluded. J. Anim. Sci. 77:1869–1874.

Breen, K. M., and F. J. Karsch. 2004. Does cortisol inhibit pulsatile luteinizing hormone secretion at the hypothalamic or pituitary level? Endocrinology 145:692–698.

D'Occhio, M. J., and D. E. Brooks. 1982. Threshold of plasma testosterone required for normal mating activity in male sheep. Horm. Behav. 16:383–394.

Harding, C. F., and H. H. Feder. 1976. Relation between individual differences in sexual behavior and plasma testosterone levels in the guinea pig. Endocrinology 98:1198–1205.

Haynes, N. B., and B. D. Schanbacher. 1983. The control of reproductive activity in the ram. Pages 431–451 in Sheep Production. Butterworths, London, UK.

Ji, W.-Z., A. H. Kaynard, K.-Y. F. Pau, D. L. Hess, W. L. Baughman, and H. G. Spies. 1989. Endogenous opiates regulate the nocturnal reduction in luteinizing hormone pulse frequency during the luteal phase of the Macaque menstrual cycle. Biol. Reprod. 41:1024–1033.

Juniewicz, P. E., B. H. Johnson, and D. J. Bolt. 1987. Effect of adrenal steroids on testosterone and luteinizing hormone secretion in the ram. J. Androl. 8:190–196.

Kenward, M. G., and J. H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53:983–997.

Matteri, R. L., J. G. Watson, and G. P. Moberg. 1984. Stress or acute adrenocorticotrophin treatment suppresses LHRH-induced LH release in the ram. J. Reprod. Fertil. 72:385–393.

Orr, T. E., and D. R. Mann. 1990. Effects of restraint stress on plasma LH and testosterone concentrations, Leydig cell LH/hCG receptors, and in vitro testicular steroidogenesis in adult rats. Horm. Behav. 24:324–341.

Orr, T. E., M. F. Taylor, A. K. Bhattacharyya, D. C. Collins, and D. R. Mann. 1994. Acute immobilization stress disrupts testicular steriodogenesis in adult male rats by inhibiting the activities of 17 alpha-hydroxylase and 17, 20- lyase without affecting the binding of LH/hCG receptors. J. Androl. 15:302–308.

Perkins, A., J. A. Fitzgerald, and G. E. Moss. 1995. A comparison of LH secretion and brain estradiol receptors in heterosexual and homosexual rams and female sheep. Horm. Behav. 29:31–41.

Perkins, A., J. A. Fitzgerald, and E. O. Price. 1992. Luteinizing hormone and testosterone response of sexually active and inactive rams. J. Anim. Sci. 70:2086–2093.

Pinckard, K. L., J. N. Stellflug, and F. Stormshak. 2000. Influence of castration and estrogen replacement on sexual behavior of female-oriented, male-oriented, and asexual rams. J. Anim. Sci. 78:1947–1953.

Rivier, C., and S. Rivest. 1991. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: Peripheral and central mechanisms. Biol. Reprod. 45:523–532.

Roselli, C. E., K. Larkin, J. A. Resko, J. N. Stellflug, and F. Stormshak. 2004. The volume of a sexually dimorphic nucleus in the ovine medial preoptic area/anterior hypothalamus varies with sexual partner preference. Endocrinology 145:478–483.

Roselli, C. E., F. Stormshak, J. N. Stellflug, and J. A. Resko. 2002. Relationship of serum testosterone concentrations to mate preferences in rams. Biol. Reprod. 67:263–268.

Sanford, L. M., J. S. D. Winter, W. M. Palmer, and B. E. Howland. 1974. The profile of LH and testosterone secretion in the ram. Endocrinology 96:627–631.

Schanbacher, B. D., and D. D. Lunstra. 1976. Seasonal changes in sexual activity and serum levels of LH and testosterone in Finnish Landrace and Suffolk rams. J. Anim. Sci. 43:644–650.

Snowder, G. D., J. N. Stellflug, and L. D. Van Vleck. 2002. Heritability and repeatability of sexual performance scores of rams. J. Anim. Sci. 80:1508–1511.

Stackpole, C. A., A. I. Turner, I. J. Clarke, G. W. Lambert, and A. J. Tillbrook. 2003. Seasonal differences in the effect of isolation and restraint stress on the luteinizing hormone response to gonadotropin-releasing hormone in the hypothalamopituitary disconnected, gonadectiomized rams and ewes. Biol. Reprod. 69:1158–1164.

Stellflug, J. N. 2002. Use of naloxone challenge to predict sexual performance of rams before the fall breeding season. Theriogenology 58:123–134.

Stellflug, J. N., and J. G. Berardinelli. 2002. Ram mating behavior after long-term selection for reproductive rate in Rambouillet ewes. J. Anim. Sci. 80:2588–2593.

Stellflug, J. N., A. Perkins, and V. A. LaVoie. 2004. Testosterone and luteinizing hormone responses to naloxone help predict sexual performance in rams. J. Anim. Sci. 82:3380–3387.

Tillbrook, A. J., B. J. Canny, M. D. Serapiglia, T. J. Ambrose, and I. J. Clarke. 1999. Suppression of the secretion of luteinizing hormone due to isolation/restraint stress in gonadectomised rams and ewes is influenced by sex steroids. J. Endocrinol. 160:469–481.

Veldhuis, J. D., M. L. Carlson, and M. L. Johnson. 1987. The pituitary secretes in bursts: Appraising the nature of glandular, secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. Proc. Natl. Acad. Sci. USA 84:7686–7690.

Veldhuis, J. D., and M. L. Johnson. 1986. Cluster analysis: A simple, versatile, and robust algorithm for endocrine pulse detection. Am. J. Physiol. 250(EM 13):E486–E493.

Welsh, T. H., Jr., T. H. Bambino, and A. J. W. Hsueh. 1982. Mechanism of glucocorticoid-induced suppression of testicular androgen biosynthesis in vitro. Biol. Reprod. 27:1138–1146.

Welsh, T. H., and B. H. Johnson. 1981. Stress-induced alterations in corticoids, progesterone, luteinizing hormone, and testosterone in bulls. Endocrinology 109:185–190.

Welsh, T. H., C. N. Kemper-Green, and K. N. Livingston. 1999. Stress and reproduction. Vol. 4, pages 662–674 in Encyclopedia of Reproduction. E. Knobil and J. D. Neill, ed. Academic Press, San Diego, CA.

Whalen, R. E., F. A. Beach, and R. E. Kuehn. 1961. Effects of exogenous androgens on sexually responsive and unresponsive male rats. Endocrinology 68:373–380.

Wilson, P. R., and K. R. Lapwood. 1979. Studies of reproductive development in Romney rams. I. Basal levels and plasma profiles of LH, testosterone and prolactin. Biol. Reprod. 20:965–970.